Flavonoid Synthesis and Antheridium Initiation in Dryopteris Gametophytes

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There is now a fairly extensive vascular crytogam flavonoid literature and there are a number of researchers actively engaged in this research field (Swain & Cooper-Driver, 1973). All vascular crytogam flavonoid work has been done on the sporophyte generation, with one exception: Laurent (1966) determined that *Blechnum brasiliense* gametophytes produced the flavonoid kaempferol, which is one of the flavonoids produced by the *B. brasiliense* sporophyte. Reasons for the paucity of information on fern gametophyte flavonoids include the easy accessibility of sporophytes and the now disreputed opinion that flavonoids, being associated with lignin synthesis, are exclusive to vascularized plant bodies. This exclusivity has been lost because flavonoids have been isolated and identified in various non-vascular plant groups: certain algal divisions, bryophytes (Swain, 1974), and fern gametophytes (Laurent, 1966).

Initially our investigation was undertaken to determine if *Dryopteris intermedia* A. Gray and *D. marginalis* A. Gray gametophytes produce flavonoids and, if so, were these the same flavonoids produced by their sporophytic counterparts (Petersen, 1976). Because of the unusual results of this first portion of the research, the inquiry was amplified to include an analysis of flavonoid content along a developmental profile of the gametophytes.

Half-strength White's minimum nutrient medium adjusted to pH 6.0 was used to culture the gametophytes. Liquid cultures were prepared by placing 0.25 g of spores in a 4 l flask and adding 2 l of nutrient solution. Separate cultures of *D. intermedia* and *D. marginalis* were grown at 22°C under 300 ft-c of illumination from cool-white fluorescent lights in a 12/12 hr diurnal cycle.

Gametophytes were harvested and assayed for flavonoids at three developmental stages: (1) pre-antheridial initiation (0 antheridia/gametophyte), (2) antheridial initiation (0 or 1 antheridia/gametophyte), and (3) post-antheridial initiation (4–6 antheridia/gametophyte).

Ten-gram samples of harvested gametophytes were immediately extracted in methanol and re-extracted repeatedly until a colorless supernatant was obtained. Concentrated extracts were spotted onto Whatman 3MM chromatography paper (42 × 55 cm). Chromatograms were developed in two dimensions employing the two standard solvent systems for the separation of flavonoids: t-butanol, acetic acid, water (3:1:1) for the first dimension and 15% acetic acid, water for the second dimension. Completed chromatograms were inspected under UV light, both in the presence and absence of NH_3. Spot color changes under both conditions were noted and R_f values determined. Spots were excised, eluted in spectral grade methanol, and UV spectral data obtained using standard procedures (Mabry

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et al., 1970). Positive determinations of isolated flavonoid aglycones were done by co-chromatographing them against authentic compounds. Quantitative scoring for flavonoid content was done by comparative visual inspection of spot intensity and size.

In the initial experiment, *D. marginalis* gametophytes were cultured for 30 days and then harvested. Because of extreme crowding, most of the gametophytes formed filaments rather than plates, and most bore a number of antheridia laterally. Flavonoids were detected in these gametophytes, and they were the same ones that occur in *D. marginalis* sporophytes *(Table 1)*.

TABLE 1. IDENTIFICATION DATA FOR FLAVONOID GLYCOSIDES OF QUERCETIN AND KAEMPFEROL FOUND IN *DRYOPTERIS INTERMEDIA* AND *D. MARGINALIS* GAMETOPHYTES AND SPOROPHYTES.

	Quercetin (A)	*Kaempferol* (B)	*Kaempferol* (C)
	CHROMATOGRAM SPOT APPEARANCE		
UV	Violet	Violet	Violet
UV/NH_3	Yellow	Yellow	Yellow
	CHROMATOGRAM SPOT R_f VALUES		
TAB	0.50	0.60	0.63
HOAc	0.44	0.56	0.45
	UV SPECTRAL DATA (λ max., nm)		
MeOH	256, 282, 306, 357	266, 292, 347	265, 302, 349
NaOMe	271, 323, 412	274, 324, 401	275, 325, 401
$AlCl_3$	270, 302sh, 362sh, 403	274, 302, 349, 395	273, 302, 348, 394
$AlCl_3/HCl$	268, 300sh, 358,	273, 302, 343, 392	273, 298, 343, 392
NaOAc	273, 415	273, 302, 381	273, 301, 375

Because of inadequate material for spectrometric analysis, new cultures of *D. marginalis* were started, and cultures of *D. intermedia* were begun to determine if they likewise produced the same flavonoids as *D. intermedia* sporophytes. After three weeks, the cultures were harvested and assayed for flavonoids. No flavonoids were detected on the chromatograms, and examination showed that the gametophytes had not produced antheridia. Therefore, more gametophytes were cultured so that flavonoid content could be analyzed at three developmental stages *(Table 2)*.

The last experiments showed that the same flavonoids are produced by the gametophytes of these two species as are produced by their respective sporophytes *(Table 1)*. They are quercetin and kaempferol glycosides. (See Mabry et al., 1970, for data comparisons and structural details.) *Dryopteris intermedia* sporophytes and gametophytes produced two flavonoids: a quercetin glycoside (Compound A) and a kaempferol glycoside (Compound B). *Dryopteris marginalis* produced these two compounds, as well as an additional kaempferol glycoside (Compound C).

The experiments *(Table 2)* also show flavonoids to be absent (−) during the pre-antheridial stage. They were first detected as faint spots (+) at the onset of antheridium formation. Flavonoid concentration is much higher (++) in the post-antheridium initiation stage than at the onset of antheridium formation, as revealed by greater intensities of spot fluorescence. Antheridium formation and

TABLE 2. FLAVONOID CONTENT OF *DRYOPTERIS* GAMETOPHYTES AT THREE DEVELOPMENTAL STAGES.

	Pre-antheridial initiation			*Antheridial initiation*			*Post-antheridial initiation*		
COMPOUNDS	*(A)*	*(B)*	*(C)*	*(A)*	*(B)*	*(C)*	*(A)*	*(B)*	*(C)*
D. intermedia	−	−	−	+	+	−	++	++	−
D. marginalis	−	−	−	+	+	+	++	++	++

− = compound not detected; + = compound detected but at a low concentration relative to ++.

flavonoid synthesis clearly are associated, but whether the two events are interrelated (cause and effect) or merely a coincidence remains to be determined. Although nothing comparable has been discovered in flowering plants, Barber (1956) identified a glucose-rhamnose glycoside of quercetin in staminate squash flowers that was absent from the pistillate flowers.

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LITERATURE CITED

BARBER, G. A. 1956. Flavonoids of staminate and pistillate squash flowers. Arch. Biochem. Biophys. 64:401–411.

LAURENT, S. 1966. Contribution à l'étude des tanins et des autres substances phénoliques hydrosolubles, élaborées par les prothalles de filicinées. Thèse Docteur l'Université de Paris.

MABRY, T. J., K. R. MARKHAM, and M. B. THOMAS. 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York, Heidelberg, and Berlin.

PETERSEN, R. L. 1976. Chemical research in the genus Dryopteris Adanson: systematics, morphogenesis, and allelopathy. Ph.D. Thesis. Rutgers, The State University, New Brunswick, New Jersey.

SWAIN, T. 1974. Biochemical evolution in plants (Chapter II). *In* M. Florkin and E. H. Stotz, eds. Comprehensive Biochemistry, vol. 29A. Elsevier, Amsterdam.

———, and G. COOPER-DRIVER. 1973. Biochemical systematics in the Filicopsida. *In* A. C. Jermy, J. A. Crabbe, and B. A. Thomas, eds. The Phylogeny and Classification of the Ferns. Bot. J. Linn. Soc. 67, Suppl. 1. Academic Press, New York and London.